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Characteristics of Rectal Carcinomas That Predict the Presence of Lymph Node Metastases: Implications for Patient Selection for Local Therapy

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Background and Objectives: It has been estimated that approximately 5% of middle and low rectal adenocarcinomas are amenable to local therapy. However, these treatment modalities are limited by their failure to identify and treat regional nodal metastases.

Methods: This study was undertaken to evaluate the role of tumor size, depth of penetration into the rectal wall, degree of histologic differentiation, DNA ploidy status, and their combination on the presence or absence of metastases in perirectal lymph nodes. Logistic regression was used to quantitatively predict the probability of positive lymph nodes.

Results: Tumor size did not correlate with the presence of nodal involvement; however, worsening degree of differentiation, increasing depth of wall penetration and aneuploidy did statistically correlate with the presence of nodal metastases. For any combination of tumor traits, aneuploidy markedly increased the probability of positive lymph nodes over that observed with diploid tumors.

Conclusions: The combination of degree of differentiation, depth of penetration, and ploidy status may be used to identify patients whose tumors may be adequately treated with local measures. For any combination of tumor traits, aneuploidy markedly increased the probability of positive lymph nodes over that observed with diploid tumors.

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KEY WORDS: **rectal cancer; nodal metastases; DNA ploidy; flow cytometry; local resection**

INTRODUCTION

It is estimated that approximately 5% of patients with rectal cancer are candidates for potentially curative local therapy [1–3]. These treatment modalities include local excision, electrofulguration, and intracavitary radiotherapy. For low rectal tumors, the gold standard is the classic Miles abdominoperineal resection. However, patients often are reluctant to accept this procedure because of its obligatory permanent colostomy [4,5]. While local therapy does preserve normal anorectal anatomy and function it does not identify or treat the regional lymphatics which may harbor subclinical metastases. Several investigators have attempted to define those characteristics of rectal tumors that may identify which patients would be appropriate candidates for local therapy. The characteristics examined include wall penetration, size, histology, gross appearance and pelvic fixation. These

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have been used with varying degrees of success. A key weakness of these traits is their uncertain ability to accurately and reliably predict nodal status [6]. Trans-anal ultrasound is fairly accurate in measuring the depth of penetration of tumors, but it is less useful in identifying nodal metastasis [7]. The addition of flow cytometric DNA analysis to the management of solid tumors over the past decade has added to the prognostic capabilities in melanomas and in solid tumors of the breast, head and neck, bladder, and colorectal cancer [8]. While its usefulness as an indicator of recurrence risk and survival has been described in some series, the role of flow cytometry in predicting preoperative nodal status in rectal carcinoma has been addressed specifically in only one study [9].

This retrospective study was undertaken in order to evaluate the prognostic value of DNA ploidy analysis, degree of differentiation, depth of wall penetration, and tumor size as predictors of nodal spread in rectal carcinoma. These data could be utilized to identify those patients who are most likely to enjoy the benefits of local therapy without sacrificing a chance for cure.

MATERIALS AND METHODS

The medical records and archival pathologic specimens of all patients undergoing abdominoperineal resection (APR) for rectal adenocarcinoma at the Loyola University Medical Center and Hines VA Hospital between July 1, 1971 and December 31, 1989 were reviewed retrospectively. Excluded were those patients who underwent pelvic exenteration, received preoperative radiation, had a tumor located more than 16 cm above the anal verge pathologically were found to have invasive carcinoma in a villous adenoma or had distant metastases. Of the 133 patients with adenocarcinoma of the rectum who fulfilled these criteria, 129 specimens (96.9%) were available for DNA flow cytometry analysis.

Charts were evaluated for age, sex, tumor location above the anal verge on pathologic examination, histology (differentiation: well, moderate, poor), size (more than 3 cm vs less than or equal to 3 cm), depth of wall penetration (TNM staging system: T1, penetration into the submucosa; T2, incomplete muscularis propria penetration; T3, penetration through muscularis propria) [10] and nodal status. Patients with distant metastases were excluded.

When available, paraffin embedded archival pathology specimens underwent DNA analysis using the flow cytometric technique of Hedley et al. [11]. The specimens were cut from tissue blocks using a microtome, and were dewaxed in xylene. Rehydration of the specimens was performed by sequential immersion in 100%, 95%, 70%, and 50% ethanol, followed by washing with distilled water. Using 0.5% pepsin solution at 37°C and pH 1.5 with vigorous vortexing, single cell suspensions were generated. Tissue sections were filtered and the nuclei washed with phosphate-buffered saline (PBS). The nuclei were then subjected to RNase treatment (1.0% solution at 37°C for 30 min) and stained with 0.005% propidium iodine for measurement using a Becton-Dickinson FacScan flow cytometer (San Jose, CA). Because of the lack of an acceptable internal standard, the first peak was routinely given the DNA index (DI) of 1.0 and was referred to as the diploid G_0/G_1 peak. All other peaks were compared to the modal channel of the diploid peak and assigned a DI relative to the first peak. An aneuploid population was considered present only when there were two or more distinct G_0/G_1 DNA peaks.

Two-tailed chi-square tests were used to define the relationship between individual tumor variables and nodal status. Logistic regression analysis was employed to analyze the role of the combination of tumor characteristics as a predictor of the presence or absence of nodal metastases. Significance was accepted at $P < 0.05$.

RESULTS

There were 96 male and 37 female patients in our study, reflecting the large number of male veterans included in this series. Ages ranged from 29 to 92 years (mean 62.5). According to the TNM classification system, there were 10 (8%) T1 tumors, 35 (26%) T2 tumors, and 88 (66%) T3 tumors.

Table I summarizes the tumor characteristics in the group of 133 patients studied here and also examines the four tumor characteristics of size, depth of penetration, histologic differentiation, and ploidy as predictors of lymph node metastases. This analysis treats each tumor characteristic as though it were an independent variable. Overall, 55 of 133 patients (41%) were node positive. Most of the rectal tumors were more than 3 cm in size. However, there was no statistically significant correlation between tumor size and nodal metastases. As the depth of tumor penetration increased there was an increased incidence of positive lymph nodes. Tumor differentiation correlated statistically with nodal disease in that the less differentiated a tumor, the greater the likelihood of nodal metastases ($P < 0.001$). Ten percent of T1 tumors, 26% (9/35) of T2 tumors and 51% (45/88) of T3 tumors were associated with early metastases. As shown in Table I, only 22 of 133 tumors were poorly differentiated, but 18 of these 22 specimens (82%) were node positive.

Of the 133 patients, ploidy status was available for all but four tumors. Of the 129 archival specimens available for DNA ploidy flow cytometry, 88 (68%) were diploid with the remaining classified as aneuploid (Table I). One-third of diploid tumors (34%) were associated with positive lymph nodes, whereas almost twice that number (59%) of the aneuploid tumors were associated with

TABLE I. Rectal Carcinoma Tumor Characteristics Versus Nodal Status

	Lymph node (%)			
	Total (%)	Negative 78 (59)	Positive 55 (41)	*P**
Size (largest diameter) (133 pts.[a])				
⩽3 cm	27 (20)	19 (70)	8 (30)	NS[b]
>3 cm	106 (80)	59 (56)	47 (44)	
Wall penetration (133 pts.)				
T1	10 (8)	9 (90)	1 (10)	
T2	35 (26)	26 (74)	9 (26)	<0.001
T3	88 (66)	43 (49)	45 (51)	
Differentiation (133 pts.)				
Well	50 (38)	42 (84)	8 (16)	
Moderate	61 (46)	32 (53)	29 (47)	<0.0001
Poor	22 (16)	4 (18)	18 (82)	
Ploidy (129 pts.)				
Diploid	88 (68)	58 (66)	30 (34)	<0.02
Aneuploid	41 (32)	17 (41)	24 (59)	

*Chi-square test, significant at $P < 0.05$.
[a]pts., patients.
[b]NS, not statistical.

positive lymph nodes. This difference was statistically significant ($P < 0.02$).

Degree of differentiation and depth of penetration correlated strongly with each other ($P < 0.001$) as did DNA ploidy and differentiation ($P < 0.05$) (data not shown in tables). This was not quite the case ($P = 0.07$) for the relationship between ploidy and depth of penetration (data not shown in tables). Thus, the four variables analyzed in Table I were not truly independent because they occurred in various combinations in each patient. Accordingly, the data were analyzed using logistic regression. Table II presents the probability of finding positive lymph nodes for all the combinations of tumor characteristics that were observed. Tumor size was excluded after an initial logistic regression demonstrated no relationship between tumor size and lymph node status. The data are grouped by degree of differentiation (well, moderate, poor). These are then broken down further by depth of tumor penetration (T1, T2, T3) for each degree of differentiation. Finally each group is broken down further into ploidy status (diploid vs. aneuploid). Table II permits the reader to assess from the logistic regression analysis the probability of positive lymph node status for all the combinations of differentiation, penetration, and ploidy. These data show that the probability of positive lymph nodes was as low as 0.06, i.e., 6% with a well-differentiated, minimally penetrating (T1), diploid tumor. At the other extreme, the probability of positive lymph nodes was 0.88, i.e., 88% with a poorly differentiated, deeply penetrating (T3), aneuploid tumor. Intermediate levels of probability were found for each of the combinations between these extremes. It is evident from these data that tumors that exhibited an increasing number of ''unfavorable'' characteristics were associated with an increasing probability of lymph node metastases, and that aneuploid tumors exhibited up to two-fold increased risk of positive lymph nodes for any given combination of histologic and penetration status.

TABLE II. Probability of Nodal Metastasis in Rectal Carcinoma by Logistic Regression

Differentiation	Penetration[a]	Ploidy[b]	Probability
Well	T1	D	0.06
	T2	D	0.11
	T2	A	0.20
	T3	D	0.18
	T3	A	0.31
Moderate	T1	D	0.21
	T2	D	0.32
	T2	A	0.50
	T3	D	0.46
	T3	A	0.64
Poor	T2	D	0.65
	T3	D	0.77
	T3	A	0.88

[a]T1, mucosal and submucosal penetration; T2, incomplete muscularis propria penetration; T3, penetration through the muscularis propria.
[b]D, diploid; A, aneuploid.

DISCUSSION

Although many mid-rectal cancers and some low rectal cancers may be treated with either low anterior resection or colo-anal anastomosis, there remain some lesions that require abdominoperineal resection for complete extirpation of the tumor and its nodal drainage. Abdominoperineal resection with its attendant permanent colostomy is sometimes perceived by the patient as worse than the cancer for which it is performed. In fact, abdominoperineal resection may overtreat some patients who do not have nodal metastases. Some tumors may be ad-

equately treated by local measures such as excision, fulguration, or radiotherapy. The size and differentiation of a rectal tumor usually are apparent preoperatively. Intrarectal ultrasound may quite accurately detect the depth of the tumor invasion, but it is less useful for revealing nodal status. A recent review of the use of intrarectal ultrasound for preoperative staging of rectal tumors demonstrated accuracy in the range of 78–91% for depth of penetration but only 62–83% for the presence of lymph node metastases [7]. Local therapy does not permit appropriate surgical staging. Similarly, the inability of local therapy to treat regional spread may predispose to increased risk of local recurrence and shortened long term survival.

Various investigators have attempted to define clinicopathologic parameters which identify lesions associated with a low incidence of regional metastases. Patients with these low-risk or ''early'' rectal carcinomas would be ideal candidates for local management with acceptable rates of disease recurrence and long term survival. Morson [12], reported that only 12% of tumors with incomplete wall penetration presented with positive lymph nodes. Based on this report and others [13–17], it has been suggested that local excision can safely be performed on tumors that do not exhibit complete wall penetration or are poorly differentiated. Some investigators have included a size restriction. Biggers et al. [14] and Hager et al. [17], recommended the use of local therapy only for lesions measuring 3 cm or less, while Gingold et al. [18], in reviewing the results of local therapy with electrofulguration, broadened this criteria to include tumors up to 4 cm. This recommendation has been generally accepted despite the fact that Greaney and associates found no relationship between tumor size and lymphatic involvement [19]. Nelson et al. [6] did a retrospective study of patients with rectal cancer treated with radical resection. More than one-half of the patients with ''favorable'' criteria (i.e., well or moderate differentiation and size $\leqslant$4 cm) were found to have positive lymph nodes. In addition, 25% of lesions with incomplete wall penetration had nodal involvement. Chang et al. [20] analyzed the DNA ploidy in rectal carcinoma treated by local therapy in an attempt to better predict long term outcome. They found that aneuploid DNA status correlated closely with subsequent aggressive clinical behavior.

Kimura et al. [9] described DNA content in terms of ploidy (diploid or aneuploid) and DNA index (DI), which they defined as the value of the mode of the aneuploid peak divided by the mode of the diploid peak. They found a significant difference in the incidence of lymph node metastases in aneuploid lesions with DI values greater than 1.5 versus diploid tumors with DI values below 1.4. They also noted that distant lymph node metastases were frequent in cases with aneuploid tumors, especially in those with a DI above 1.5, whereas lymph node metastases were limited to adjacent lymph nodes in diploid tumors. Finally, they noted that the incidence of local recurrences was significantly higher in cases with aneuploid tumors with DI values of greater than 1.5 versus diploid tumors or aneuploid tumors with DI values below 1.4.

TABLE III. Rectal Carcinoma Percentage of Tumors With Lymph Node Metastasis

	T2 (%)	T3 (%)
Nelson et al. [16]	22	66
Minsky et al. [21]	28	36
Kimura et al. [9]	22	44
Saclarides et al. [22]	35	59
Present series	26	51

In the present study of resected tumors, various tumor characteristics and their combinations were used to judge whether a rectal tumor would have been suitable for local treatment. These data agree with those of Graham [16] and Greaney [19] that indicate size, by itself, does not accurately predict the presence or absence of positive lymph nodes since almost 30% of our patients with tumors less than or equal to 3 cm had lymphatic involvement.

The depth of wall penetration did strongly correlate with node status. One patient of 10 (10%) with only submucosal penetration (T1) had lymphatic metastases, and 10 of 45 patients (22%) with tumors incompletely penetrating the rectal wall (T2) had positive lymph nodes. Nelson et al. [6], Minsky et al. [21], Saclarides et al. [22], and Kimura et al. [9], reported a similar incidence of nodal involvement despite incomlete rectal wall penetration (Table III). This contrasts with Morson's original report of a 12% incidence of nodal involvement with incomplete penetration [12]. This value is approximately one-half that found in more recent studies. While worsening differentiation predicted an increased incidence of lymphatic metastases, even the ''favorable'' histologic grades (well and moderate) had a 33% incidence of regional metastases. It is clear, therefore, that standard pathologic assessment is of limited value in successfully identifying those patients who might be spared radical surgery.

By using ploidy in combination with other traditional criteria one may be able to more successfully predict the likelihood that nodal spread has occurred. The data presented in Table II summarize the contributions of all the tumor characteristics in combination to predict the probability of positive lymph nodes. It is noteworthy that, for any combination of degree of differentiation, tumor size, and depth of penetration, aneuploidy increased the probability of positive lymph nodes 5-22 percentage points over that observed with diploidy. In many cases, this doubled the risk of having positive lymph nodes.

Operative mortality from abdominoperineal resection ranges from 2.0% to 6.5%. In addition, up to 61% of patients have postoperative complications, most frequently involving micturition, sexual function, the perineal wound, or the stoma [23,24]. The present data have clinical value, in that they allow us to project more accurately the probability of the presence of nodal metastases. In choosing the modality of treatment, one might accept a higher probability of metastases which would go untreated with a local procedure in an elderly patient with significant comorbidity while such a risk would be unacceptable in an otherwise healthy patient and the radical operation would be the recommended treatment modality.

CONCLUSIONS

Degree of wall penetration, histologic differentiation, and ploidy status all influence the probability that a given patient with a rectal tumor will have metastases to local lymph nodes. The present data, utilizing logistic regression, permitted quantitative prediction of positive lymph node metastases. These data suggest that those patients with a favorable combination of characteristics may be candidates for local treatment, whereas patients at high risk would be better served by more radical surgery in order to sample and treat the lymphatic drainage.

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